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(54) Title: 1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

(57) Abstract

A method of using 1,2,4-benzotriazine oxides as radiosensitizers and selective cytotoxic agents is disclosed. The compounds are shown to specifically radiosensitive hypoxic tumor cells and are additionnally disclosed to be useful as specific cytotoxic agents for these cells.

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1,2,4-BENZOTRIAZINE OXIDES AS RADIOSENSITIZERS AND SELECTIVE CYTOTOXIC AGENTS

The herein application is a continuation-in-part of U.S. Application Serial No. 911,906, filed 25 September 1986.

Reference to Government Grant or Contract

The invention described herein was made in the course of work under grant or contract from the Department of Health and Human Services. The Government has certain rights in this invention.

Technical Field

The invention relates to cytotoxic agents and radiotherapy effective against hypoxic cells. Specifically, the invention relates to selectively killing tumor cells and to sensitizing tumor cells to radiation using 1,2,4-benzotriazine oxides.

Background Art

Hypoxic cell radiosensitizers are compounds that selectively increase the sensitivity of hypoxic cells to destructive radiation. Cytotoxins which have enhanced activity under hypoxic conditions also provide a means for selective destruction of cells under low oxygen pressure. This specificity for hypoxic cells is important because it is tumors that are typically characterized by such cells. Virtually all tumors which

are present as solid masses contain these cells, while normal cells generally have an adequate supply of oxygen. Accordingly, anti-tumor agents can be made selective for tumors by virtue of high activity under hypoxic conditions, and radiation can be employed more effectively in the presence of these sensitizers.

Of course, the use of radiation treatment to destroy tumor cells is only practical if damage to the surrounding normal tissue can be minimized or avoided. The effects of radiation are enhanced by the presence of oxygen, and it is established that as the dose of radiation is increased, the effectiveness of the radiation in destroying target cells is enhanced most dramatically when oxygen is present. Therefore, selectivity for tumor cells toward radiation is difficult to achieve -- normal cells, in view of their oxygen supply, are generally more susceptible to radiation than the target tumor cells. It is therefore desirable to provide a means of sensitizing tumor cells, but not the surrounding tissue, to radiation treatment. One solution would be to increase the supply of oxygen to these tumor cells. This, however, has proved difficult to do.

Various heterocyclic compounds and in particular those with oxidized nitrogen moieties, have been used to radiosensitize hypoxic tumor cells. Indeed, it has been postulated that the oxidized nitrogen functionality is responsible for this activity. Nitroimidazoles, particularly misonidazole (MIS) and metronidazole have been studied extensively, and MIS is commonly used as a standard in in vitro and in vivo tests for radiosensitizing activity. (See, e.g., Asquith, et al, Radiation Res (1974) 60:108-118; Hall,

et al, Brit J Cancer (1978) 37: 567-569; Brown, et al, Radiation Res (1980) 82:171-190; and U.S. patent 4,371,540. The radiosensitizing activities of certain 1-substituted 3(5)-nitro-s-triazoles and of various quinoxaline-1,4-dioxide derivatives have also been disclosed.

In addition, US Serial Nos. 730,761, filed 3 May 1985, and 788,762, filed 18 October 1985 assigned to the same assignee and incorporated by reference disclose a group of radiosensitizers that do not contain oxidized nitrogen -- the substituted benzamides and nicotinamides and their thio analogs. These compounds, nevertheless, are radiosensitizers. It is important to distinguish the ability to sensitize hypoxic cells selectively, for instance, by enhancing their oxygen supply, from another mechanism commonly encountered for "sensitizing" cells: inhibition of the enzyme poly(ADP-ribose)polymerase, which is believed to be essential in the repair of irradiated cells after radiation. This repair mechanism is operative in both hypoxic tumor cells and in normal cells. Hence, administration of "radiosensitizers" which operate according to this latter mechanism does not accomplish the desired purpose of selectively sensitizing the target tumor cells.

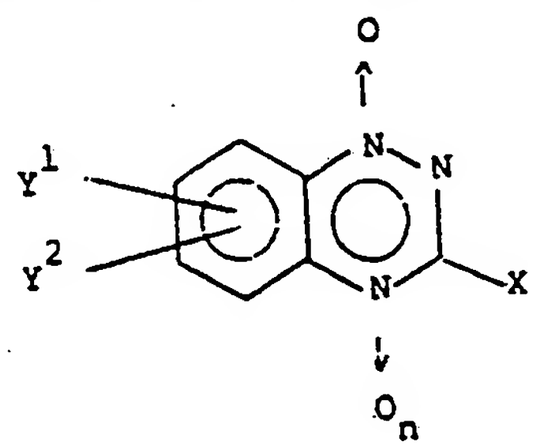
A group of compounds which has not previously been suggested for use in either selectively killing hypoxic cells or in radiosensitizing such cells is 3-amino-1,2,4-benzotriazine 1,4-di-N-oxide and related compounds. Related US patents 3,980,779; 3,868,371; and 4,001,410 disclose the preparation of a group of these compounds and their use as anti-microbial agents, particularly by addition of these materials to livestock fodder. US patents 3,991,189 and 3,957,799 disclose

derivatives of these compounds bearing substituents on the nitrogen of the 3-amino group. These compounds also have anti-microbial activity.

The present invention provides additional compounds which specifically radiosensitize hypoxic cells in vitro and which, furthermore, are directly cytotoxic to hypoxic cells both in vitro and in vivo. Therefore, administration of these compounds prior to or following radiation treatment of tumors selectively kills the hypoxic (tumor) cells which survive the radiation dose. Both the ability of these compounds to radiosensitize hypoxic cells in vitro and especially their ability to selectively kill hypoxic cells directly are unexpected properties of these compounds.

Disclosure of the Invention

The invention provides a valuable addition to the group of compounds currently available as selective radiosensitizers and selective cytotoxic agents for hypoxic tumor cells. Some of the compounds useful in this regard are known compounds, others are novel. One aspect of the invention, therefore, is a method of radiosensitizing or selectively killing hypoxic tumor cells with a compound of the formula:



wherein X is H, hydrocarbyl (1-4C), OH, OR, NH_2, NHR or NR_2 where each R is independently an alkyl of 1-4 carbon atoms, an amide, or a morpholino moiety

and may further be substituted with hydroxy, alkoxy, amino, or halogeno substituents;

wherein n is 0 or 1; and

Y^1 and Y^2 are independently either H, halogeno, hydrocarbyl (1-14C) including cyclic and unsaturated hydrocarbyl, optionally substituted with 1 or 2 substituents selected from the group consisting of halogeno, hydroxy, epoxy, alkoxy, alkylthio, amino (including morpholino), acyloxy, acylamido and their thio analogs, alkylsulfonyl, alkylphosphonyl, carboxy, alkoxycarbonyl, carbamyl or alkylcarbamyl, and wherein the hydrocarbyl can optionally be interrupted by a single ether (-O-) linkage, or wherein Y^1 and Y^2 are independently either NHR', O(CO)R', NH(CO)R', O(SO)R', or O(POR)R' in which R' is a hydrocarbyl optionally substituted as defined above.

The compounds of the invention, therefore, are the mono- or dioxides of optionally substituted 1,2,4-benzotriazine which may contain a hydrocarbyl (1-4C), hydroxyl or amino group, either substituted or unsubstituted, in the 3 position. While all of the compounds defined by Formula 1 are generally effective as radiosensitizers, only compounds unsubstituted at the 3-position or having a 3-amino or 3-hydrocarbyl (1-4C) substituent (i.e., X=H, hydrocarbyl (1-4C), NH_2, NHR or NR_2 with R as defined above) and which are di-N-oxides (n=1) are effective cytotoxic agents.

Certain of the compounds encompassed by Formula 1 are already known in the art as being useful for other purposes; other compounds are novel. The novel compounds encompassed by the present invention and which may be prepared by methods disclosed herein include compounds represented by the formula above, in

the following three classes: I. X is OH, OR, or NR_2 with R as defined above, n is 0 or 1, and Y^1 and Y^2 are as defined above; II. X is NH_2 or NHR with R as defined above, n is 0, and Y^1 and Y^2 are as defined above; III. X is NH_2, n is 1, and Y^1 and Y^2 are as defined above but not halogeno, saturated alkyl (1-6C) unsubstituted or halogen-substituted, alkoxy (1-6C), carbamyl, carboxy or carboalkoxy (1-6C); IV. X is H or hydrocarbyl (1-4C), n is 1, and Y^1 and Y^2 are as defined above, with the proviso that when Y^1 and Y^2 are H, X is other than methyl.

Brief Description of the Drawings

Figures 1A, 1B and 1C show the selective cytotoxicity of 3-amino-1,2,4-benzotriazine 1,4-dioxide for hypoxic cells derived from hamster, mouse and human tissues.

Figure 2 shows the *in vivo* efficacy of 3-amino-1,2,4-benzotriazine 1,4-dioxide in enhancing the killing of tumor cells when combined with radiation.

Figure 3 shows the killing of tumor cells *in vivo* by 3-amino-1,2,4-benzotriazine 1,4-dioxide when the tumor has been made hypoxic by the intraperitoneal administration of the antihypertensive drug hydralazine.

Modes of Carrying Out the Invention

A. The Compounds Useful in the Invention

The compounds useful in radiosensitizing hypoxic tumor cells as described herein are derivatives of 1,2,4-benzotriazine oxide.

The hydrocarbyl group represented by Y^1 or Y^2 may contain 1-14 carbon atoms, may be saturated or

unsaturated, cyclic or acyclic, and may optionally be interrupted by a single ether linkage. Thus, the unsubstituted form of Y^1 or Y^2 can be, for example, methyl, ethyl, n-propyl, s-butyl, n-hexyl, 2-methyl-n-pentyl, 2-ethoxyethyl, 3-(n-propoxy)-n-propyl, 4-methoxybutyl, cyclohexyl, tetrahydrofurfuryl, furfuryl, cyclohexenyl, 3-(n-decyloxy)-n-propyl, 4-methyloctyl, 4.7-dimethyloctyl, and the like.

The hydrocarbyl may be substituted with one or two substituents as follows: The halogeno substituents are fluoro, chloro, bromo, or iodo. The alkoxy substituents represented by OR' may contain 1 to 4 carbon atoms, and include, for example, methoxy, n-propoxy, and t-butoxy. The amino substituent may be NH_2, NHR or NR_2, where each R is independently an alkyl of 1-4 carbons or a morpholino moiety. R may optionally be substituted with 1-2 hydroxy, alkoxy, amino, or halogeno substituents.

The acyloxy and acylamido groups are represented by R'COO- and R'CONH-, respectively, where R' contains 1-4 carbons, and their thio analogs are represented by R'CSO- and R'CSNH-. Alkyl sulfonyl and alkyl phosphonyl are, respectively, $R'SO_2$ and R'P(OR')O- wherein each R' is independently as above defined. Carboxy is the group -C(O)OH; alkoxycarbonyl is -C(O)OR'; carbamyl is $-C(O)NH_2$; and alkylcarbamyl is -C(O)NHR'.

Where X is OH, of course, the compounds may also be prepared and used as the pharmaceutically acceptable salts formed from inorganic bases, such as sodium, potassium, or calcium hydroxide, or from organic bases, such as caffeine, ethylamine, and lysine.

When X is NH_2, pharmaceutically acceptable acid addition salts may be used. These salts are those with inorganic acids such as hydrochloric, hydrobromic or phosphoric acids or organic acids such as acetic acid, pyruvic acid, succinic acid, mandelic acid, p-toluene sulfonic acid, and so forth. (Amino substituents on the hydrocarbyl side chain can also, of course, be converted to salts.)

The 1,2,4-benzotriazine may be used as the mono- or dioxide. Either the 1-nitrogen of the triazino ring may be oxidized, or both the 1-and 4-nitrogens may be oxidized.

Specific particularly preferred compounds which are useful in the radiosensitization and cytotoxic procedures of the invention include

3-hydroxy-1,2,4-benzotriazine 1-oxide;
3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
3-amino-1,2,4-benzotriazine 1-oxide;
3-amino-1,2,4-benzotriazine 1,4-di-oxide;
6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-methoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-methoxy-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-methoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-ethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1-oxide;
6(7)-ethoxy-3-amino-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[4-acetamido-n-butanoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;
6(7)-[4-acetamido-n-butanoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;
6(7)-[4-acetamido-n-butanoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[4-acetamido-n-butanoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[1-(2,3-dihydroxy)propoxy]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4-benzotriazinel-oxide;

6(7)-[(2-furyl)methylamino]-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-[(2-furyl)methylamino]-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-[(2-furyl)methylamino]-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-(2-methoxyethylamino)-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine 1-oxide;

6(7)-(2-methoxyethylamino)-3-amino-1,2,4-benzotriazine 1,4-dioxide;

6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine 1-oxide;

6(7)-carbethoxymethoxy-3-hydroxy-1,2,4-benzotriazine 1,4-dioxide;

6(7)-carbethoxymethoxy-3-amino-1,2,4-benzotriazine 1-oxide;